CTX-M-15-non-ST131 *Escherichia coli* isolates are mainly responsible of faecal carriage with ESBL-producing Enterobacteriaceae in travellers, immigrants and those visiting friends and relatives

Corresponding author: A. Valverde, Centro de Vigilancia Sanitaria Veterinaria (VISAVET), Avda. Puerta de Hierro s/n, 28040, Madrid, Spain
E-mail: aranchavalverde@gmail.com
The first two authors contributed equally to this article, and both should be considered first author.

A. Valverde[1,2], M.-C. Turrientes[3,4], F. Norman[5,6], E. San Martín[3], L. Moreno[5,6], J. A. Pérez-Molina[5,6], R. López-Vélez[5,6] and R. Cantón[2,3]

1) *Centro de Vigilancia Sanitaria Veterinaria (VISAVET), Universidad Complutense, Madrid, Spain,* 2) *Red Española de Investigación en Patología Infecciosa (REIPI), Madrid, Spain,* 3) *Servicio de Microbiología, Hospital Universitario Ramón y Cajal and Instituto Ramón y Cajal de Investigaciones Sanitarias (IRYCIS), Madrid,* 4) *Centro de Investigación Biomédica en Red Epidemiología y Salud Pública (CIBERESP), Madrid, Spain,* 5) *Unidad de Medicina Tropical, Servicio de Enfermedades Infecciosas, Hospital Universitario Ramón y Cajal, Madrid, Spain and* 6) *Red de Investigación Cooperativa en Enfermedades Tropicales (RICET), Madrid, Spain*

Abstract

Prevalence of extended-spectrum β-lactamases (ESBL) and/or carbapenemase-producing Enterobacteriaceae (EPE and CPE) in stool samples from 75 travellers, 8 people visiting friends and relatives and 3 immigrants who had travelled or came from tropical or subtropical areas was determined. Thirty-one per cent (27/86) of the subjects were faecal carriers of EPE, and 37 EPE isolates were recovered (36 *Escherichia coli,* 1 *Klebsiella pneumoniae*). CTX-M-15 was the most prevalent enzyme (64.8%) mainly associated with *E. coli* belonging to phylogroup A and sequence type complex 10. Most of the ESBL-positive travellers (50%) had visited countries from Asia.

Keywords: CTX-M-15, faecal carriage, immigrants, non-ST131 *Escherichia coli*, travellers, VFR
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High rates of intestinal colonization with extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae (EPE), in particular *Escherichia coli*, have been reported from many regions, with CTX-M-15 being the most widespread and prevalent variant [1]. In the last few years, carbapenemase-producing Enterobacteriaceae (CPE) have been increasingly reported, with prevalence varying among countries [2]. International travel has been previously associated with EPE carriage and infection [3,4], but studies about the prevalence of EPE and CPE in travellers are scarce.

We characterized and determined the prevalence of EPE and CPE in faecal samples from 86 subjects returning from tropical or subtropical areas and who attended our Tropical Medicine Unit at Ramón y Cajal University Hospital (Madrid, Spain) from September 2011 to May 2012. Written informed consent was obtained, and subjects filled out a standardized questionnaire about clinical and epidemiological data. Inclusion criteria were not receiving antibiotics in the 3 months before medical consultation and time elapsed from arrival to medical consultation was less than 3 months. The study was approved by our Hospital Ethical Committee (reference 265/10).

Each patient was classified as one of the following: 1) traveller (including tourists, backpackers, aid workers, and workers), 2) immigrants or 3) people visiting friends and relatives (VFR)—that is, people who had travelled to their country of origin to visit their friends and family. Stools of posttravel specimens were collected within 3 days of consultation and were screened using MacConkey agar supplemented with ceftazidime and cefotaxime (2 mg/L) for EPE and with Supercarba medium for CPE [5]. One isolate per morphotype was selected for further studies. Bacterial identification and susceptibility patterns were determined by the WIDER system (Fco. Soria-Melguizo, Spain). MICs were interpreted following the EUCAST criteria, or in their absence by using the corresponding cutoff values (http://www.eucast.org).

Bacterial identification was confirmed by matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (Bruker Daltonics, Germany). ESBL and carbapenemase production were confirmed by double-disk synergy and CarbaNP tests, respectively [6]. ESBL genes (*TEM, SHV* and *CTX-M*) were

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identified by polymerase chain reaction (PCR) assays and further sequencing [7]. ESBL variants were assigned using data available online (http://www.ncbi.nlm.nih.gov/, http://www.lahey.org/studies). All ESBL-producing *E. coli* isolates were typed by pulsed-field gel electrophoresis using the PulseNet Europe protocol (http://www.pulsenetinternational.org). Clonal relatedness was established using 80% similarity criteria. Phylogenetic groups among ESBL-producing *E. coli* were identified by multiplex PCR [8]. Multilocus sequence typing was performed, and the corresponding sequence types (STs) were assigned for *E. coli* isolates (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli) and *Klebsiella pneumoniae* isolates (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html). Data were expressed as median (interquartile range), and categorical variables were compared by the chi-square test. Probability values were two-tailed; a p value of $\leq$0.05 was considered to be statistically significant.

A total of 86 subjects were enrolled onto the study: 75 (87.2%) travellers, 8 (9.3%) VFR, and 3 (3.5%) immigrants. All had travelled to or come from countries of Africa (30, 34.9%), Latin America (30, 34.9%) or Asia (26, 30.2%). All epidemiological data are shown in Table 1. The most common reason for travel was tourism (n = 46), followed by aid work (n = 28). Overall, 27 subjects (31.4%) were faecal carriers of EPE, with 24 (88.9%) of them travellers, 2 (7.4%) VFR, and 1 (3.7%) immigrant. No faecal carriage with CPE was detected, although its association with international travel has been reported [9]. The main limitation of our study is the impossibility of knowing the pretravel EPE carriage, as our Tropical Medicine Unit only attends subjects after travelling, and we cannot fully ensure that the intestinal colonization by EPE occurred in the destination area. Nevertheless, considering previous studies in Spain that have showed a prevalence of approximately 7% to 10% of EPE faecal carriage in the community [7,10], we assumed that the rate in our population would be close to this value. The percentage found in our study (31.4%) is much higher than what we expected and is similar to that recently observed among healthy travellers from the Netherlands and Sweden (31.2%) [11,12].

TABLE 1. Epidemiological characteristics of ESBL faecal carriers and non-faecal carriers

Characteristic	ESBL	No ESBL	Overall
Reason for travel			
Traveller	24	51	75
Tourism	10	29	39
Backpacker	2	5	7
Aid worker	12	16	28
Job		1	1
VFR	2	6	8
Immigrant	1	2	3
Origin			
Africa	6	24	30
South America	6	8	14
Central America and the Caribbean	3	20	23
Southeast Asia, India and Nepal	12	14	26
Symptoms			
Acute diarrhoea	15	21	36
Chronic diarrhoea	2	6	8
Abdominal pain	1	3	4
Fever	5	11	16
Asymptomatic	4	20	24
Age (years)	33 (29–38)	34 (14–70)	33.5 (29–40)
Sex			
Male	10	28	38
Female	17	31	48
Time before consultation (days)	7 (3–15)	15 (11–15)	15 (7–15)
Travel duration (days)	27.5 (18–90)	22.5 (14–70)	23 (15–90)

ESBL, extended-spectrum β-lactamase; VFR, visiting friends and relatives.
Data are expressed as numbers or as median (range).

The most common reason among EPE faecal carriers for travel was aid work (n = 12) and tourism (n = 10), but no statistical significance was found for reason for travel (Tables 1 and 2). To our knowledge, this is the first time that aid work and migrants appear to be associated with the acquisition of EPE. The most visited destinations by ESBL-positive travellers were Asia (Southeast Asia, India and Nepal) (12/24, 50%), previously described as a high-risk travel area for acquisition of these organisms [4,11,12]. Other destinations found in our series were South America (4/24; 16.7%) and Central America (3/24; 12.5%), which are areas of moderate risk [4,12,13]. The two VFRs returned from South America, and the single immigrant came from Africa. No statistical significance was found among ESBL faecal carriage and geographic area of procedence.

The most frequent reason for medical consultation of the ESBL faecal carriers was acute diarrhoea (three or more non-formed stools per day for less than 2 weeks), which was present in 55.5% (15/27) of the subjects. Only 14.8% (4/27) patients were asymptomatic at consultation (Tables 1 and 2). No statistical significance was found among EPE carriage and symptoms. However, EPE colonization has been demonstrated in patients with traveller's diarrhoea in studies from Europe and Canada [3,13–15].

Thirty-seven EPE isolates were recovered: 36 *E. coli* (97.3%) and one *K. pneumoniae* (2.7%). Molecular characterization of these isolates is shown in Table 2. CTX-M enzymes (97.3%) were the most prevalent ESBL type, as previously described [14,15]. In our series, CTX-M-15 (63.2%) was the most prevalent enzyme and was associated with all continents, followed by CTX-M-14 (13.2%), which was found in travellers from South America and Southeast Asia. These findings emphasize the global distribution of CTX-M-15 [14,16]. Interestingly, some ESBLs were particularly associated with specific areas: CTX-M-27 and CTX-M-32 were only found in travellers from the Caribbean, CTX-M-55 and CTX-M-65 from South America, and CTX-M-2 from Southeast Asia. Nevertheless, CTX-M-27 has been previously found in Asia [12] and CTX-M-55 in food of animal origin in China and in travellers from Asia [12,17].

TABLE 2. Epidemiological characteristics of faecal carriers and corresponding ESBL-producing Enterobacteriaceae

Microorganism	ESBL	Phylogenetic group[a]	ST (CC)	Pulsotype	Individual	Individual category	Country	Chief Complaint (CC)	Associated resistances
E. coli	CTX-M-15	A	ST167 (STC10)	EC5	1	Traveller (AW[b])	Sudan	Fever	Sul W
			ST10 (STC10)	EC6	1	Traveller (AW)	Sudan	Fever	G T Cip Sxt Sul W
			ST617 (STC10)	EC2	2	Traveller (AW)	Ethiopia	Acute diarrhoea	G T Ak Cip Sxt Sul W
			ST44 (STC10)	EC7	3	Traveller (AW)	Guatemala	Acute diarrhoea	G T Cip Sxt Sul W
			ST48 (STC10)	EC13	4	Traveller (TOU)	India	Acute diarrhoea/ Fever syndrome	G T Sxt Sul W
			ST656 (STC10)	EC11	5	VFR	Venezuela	Acute diarrhoea	Nal Cip Sxt W
			ST46 (STC46)	EC36	6	Traveller (AW)	India	Acute diarrhoea	G T Nal Cip Sxt
			ST1139 (STC46)	EC33	7	Traveller (TOU)	Nepal	Fever	Cip
	CTX-M-15		ST2711	EC12	8	Traveller (TOU)	Cambodia	Fever	G T Nal Cip Sxt Sul W
	CTX-M-15	B2	ST131	EC15	9	Migrant	Nigeria	Asymptomatic	G T K Nal Cip Sxt Sul W
				EC8	10	Traveller (AW)	Bolivia	Acute diarrhoea	Sul
				EC9	10	Traveller (AW)	Bolivia	Acute diarrhoea	G T Nal Cip Sul
			ST2252	EC22	11	Traveller (AW)	Indonesia	Acute diarrhoea	G T Nal Cip Sxt
			ST361	EC24	12	Traveller (TOU)	Kenya	Asymptomatic	Nal Cip Sxt
	CTX-M-15	F	ST648	EC32	14	Traveller (AW)	Angola	Fever	G T Nal Cip Sxt
				EC16	15	Traveller (AW)	India	Acute diarrhoea/Abdominal pain	G T Ak Nal Cip Sxt Sul W
				EC17	15	Traveller (AW)	India	Acute diarrhoea/Abdominal pain	G T Nal Cip Sxt Sul W
				EC1	16	Traveller (BAC)	India	Acute diarrhoea	G T Ak Nal Cip Sxt Sul W
	CTX-M-15	D	ST38 (STC38)	EC10	17	Traveller (AW)	Cambodia	Chronic diarrhoea	T Nal Cip Sul
			ST2914	EC28	18	Traveller (TOU)	India/Nepal	Acute diarrhoea/Abdominal pain	Cip Sxt
			ST405 (STC405)	EC31	13	Traveller (AW)	Mali	Asymptomatic	G T Nal Cip Sxt
			ST507	EC14	9	Migrant	Nigeria	Asymptomatic	T Ak Nal Cip Sxt Sul W
	CTX-M-15	E	ST485	EC29	18	Traveller (TOU)	India/Nepal	Acute diarrhoea/Abdominal pain	Nal Cip
	CTX-M-32	A	ST10 (STC10)	EC3	19	Traveller (TOU)	Cuba	Acute diarrhoea	Nal Cip Sxt Sul W
				EC4	19	Traveller (TOU)	Cuba	Acute diarrhoea	Cip Sxt Sul W
	CTX-M-14	A	ST48 (STC10)	EC34	20	Traveller (TOU)	Thailand	Acute diarrhoea/Fever	Nal Cip Sxt
			ST178 (STC10)	EC35	20	Traveller (TOU)	Thailand	Acute diarrhoea/Fever	Cip Sxt
	CTX-M-14		ST43 (STC10)	EC27	21	VFR	Ecuador	Abdominal pain	Sxt
	CTX-M-14	D	ST38 (STC38)	EC18	22	Traveller (AW)	Cambodia	Chronic diarrhoea	G Nal Cip Sxt
	CTX-M-27	B2	ST131	EC25	23	Traveller (TOU)	Dominican Republic	Acute diarrhoea/Fever	Nal Cip Sxt
			ST1193	EC26	23	Traveller (TOU)	Dominican Republic	Acute diarrhoea/Fever	Nal Cip Sxt
	CTX-M-55	A	ST10 (STC10)	EC20	24	Traveller (BAC)	Peru	Acute diarrhoea	Nal Cip Sxt
	CTX-M-65	E	ST3057	EC19	25	Traveller (AW)	Bolivia	Asymptomatic	G T Sxt
	CTX-M-65	B1	ST602 (STC446)	EC30	26	Traveller (TOU)	Colombia	Acute diarrhoea/Abdominal pain	Nal Cip
	CTX-M-2	A	ST554 (STC10)	EC21	11	Traveller (AW)	Indonesia	Acute diarrhoea	G T Nal Cip Sxt
	SHV-type		ST23 (STC23)	EC23	27	Traveller (TOU)	Philippines	Fever syndrome	Nal Cip
K. pneumoniae	CTX-M-15	--	ST307	KP1	9	Migrant	Nigeria	Asymptomatic	G T Nal Cip Sxt Sul W

[a]Only for Escherichia coli. BAC: Backpacker; AW: Aid Work; TOU: Tourist; VFR: visiting friends and relatives.
[b]Aid work includes: Missionary/Volunteer/Researcher. G: Gentamicin; T: Tobramicin; Ak: Amikacin; Nal: Nalidixic Acid; Cip: Ciprofloxacin; Sxt: Trimethoprim/Sulfamethoxazole; Sul: Sulfonamide; W: Trimethoprim.

Pulsed-field gel electrophoresis and phylogenetic analysis revealed 36 different *E. coli* pulsotypes (EC1 to EC36) belonging to A (47.2%, *n* = 17), B2 (19.4%, *n* = 7), D (13.9%, *n* = 5), F (11.1%, *n* = 4), E (5.6%, *n* = 2) and B1 (2.8%, *n* = 1) phylogroups. The most prevalent STs identified were ST10 (10.8%; *n* = 4), ST131 (10.8%; *n* = 4) and ST648 (10.8%; *n* = 4). Nineteen isolates (52.8%) were assigned to a known ST complex (STC); most of them belonged to STC10 (12/19) (Table 2). All the isolates belonging to STC10 were classified as phylogroup A, a complex of mainly non-virulent strains [18], and was the most prevalent STC in the multilocus sequence typing database. Moreover, STC10 has been described in Spain as a usual complex among ESBL-producing *E. coli* isolates [19], suggesting its involvement in the global expansion of ESBL enzymes. ST131 was associated with B2 *E. coli* lineage and was found in travellers returning from different countries (Bolivia, Dominican Republic and Nigeria); three carried CTX-M-15 ESBL. The low prevalence of this clone is in concordance with other studies that analysed the colonization of returning travellers [14,20]. The presence in our study of ST648 *E. coli*–producing CTX-M-15 linked to phylogroup F is noteworthy. Recently, it has been described in companion animals and horses, with a virulence profile that is an alert for the possibility of global dissemination of this clone [21].

Coresistance to non-β-lactam antimicrobial drugs was common in the EPE isolates; 84.5% (32/37) were resistant to ciprofloxacin, 78.4% (29/37) to trimethoprim-sulfamethoxazole, 67.6% (25/37) to nalidixic acid, 51.4% (19/37) to tobramycin, 48.6% (18/37) to gentamicin and 10.8% (4/37) to amikacin (Table 2). Differences in resistance between nalidixic acid and ciprofloxacin could be associated with expression of plasmid-mediated, quinolone-resistant determinants [22].

In summary, a high rate of intestinal colonization with EPE among travellers, immigrants and VFR returning to Spain, especially from Southeast Asia and India, was observed. Our findings support the role of international travel in the acquisition of these organisms, which may increase the risk of dissemination and emphasizes the importance of surveillance studies to improve the understanding of their global epidemiology. Although our results indicate that most of the colonization is due to CTX-M producers, especially CTX-M-15, as others have pointed out [13,14], the dissemination of ESBL is associated mainly with non-ST131 clones. This contrasts with data from other studies and with the global dissemination of ST131 *E. coli* isolates worldwide [23], suggesting that other clones are also involved in the dissemination of CTX-M enzymes.

Transparency declaration

All authors report no conflicts of interest relevant to this article.

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